

CLINICAL SIGNIFICANCE OF THE LEVELS OF THE GINGIVAL CREVICULAR FLUID VOLUME AND LEPTIN DURING ORTHODONTIC TOOTH MOVEMENT

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Abstract

It is assumed that leptin has a role in protecting gingival tissues, leptin stimulates the immune system and enhances bone formation by acting directly on osteoblasts. As periodontal disease progresses, the protective role of leptin on the gingiva is lost owing to a decrease in the leptin level. During orthodontic tooth movement, the early response of periodontal tissues to mechanical stress is an acute inflammatory reaction. Study of leptin therefore is a useful guide to determine its relationship with tooth movement in both tension and pressure sites and the role of this cytokine in controlling the local inflammation around the tooth. Detection of the leptin level in GCF at sites under orthodontic movement had been tested and it was found that the concentration of leptin in GCF is decreased by orthodontic tooth movement.

The present study was planned in Department of Periodontology, Manipal College of Dental Sciences Manipal. Total 20 cases were evaluated in the present study. The 10 cases were enrolled in Group A as study cases and 10 cases were enrolled in control cases. The selected 10 patients were bonded with fixed appliance 0.022" PEA bracket slot, MBT prescription. The maxillary right canines (control tooth, CT) were not bonded with the bracket. The initial wire will be 0.016" NiTi wire. The distalisation force were applied on the left canine (test tooth, TT) using 0.010 SS lace backs.

Significantly decreased levels of leptin concentration might result from the presence of inflammation adjacent to the teeth undergoing movement. It has been shown previously that orthodontic tooth movement may therefore show local traits of a damage/ repair process with inflammation-like reactions: high vascular activity, many leukocytes and macrophages, and involvement of the immune system.

Keywords: Leptin levels, periodontal disease activity, orthodontic tooth movement, inflammatory markers

Introduction

Gingival and periodontal pockets (also informally referred to as gum pockets [1]) are dental terms indicating the presence of an abnormal depth of the gingival sulcus near the point at which the gingival tissue contacts the tooth. The interface between a tooth and the surrounding gingival tissue is a dynamic structure. [2] The gingival tissue forms a crevice surrounding the tooth, similar to a miniature, fluid-filled moat, wherein food debris, endogenous and exogenous cells, and chemicals float. The depth of this crevice, known as a sulcus, is in a constant state of flux due to microbial invasion and subsequent immune response. Located at the depth of the sulcus is the epithelial attachment, consisting of approximately 1 mm of junctional epithelium and another 1 mm of gingival fiber attachment, comprising the 2 mm of biologic width naturally found in the oral cavity. The sulcus is literally the area

of separation between the surrounding epithelium and the surface of the encompassed tooth.

A gingival pocket presents when the marginal gingiva experiences an edematous reaction, whether due to localized irritation and subsequent inflammation, systemic issues, or drug induced gingival hyperplasia. Regardless of the etiology, when gingival hyperplasia occurs, greater than normal (the measurement in a pre-pathological state) periodontal probing measurements can be read, creating the illusion that periodontal pockets have developed. This phenomenon is also referred to as a false pocket or pseudopocket. The epithelial attachment does not migrate, it simply remains at the same attachment level found in pre-pathological health. The only anatomical landmark experiencing migration is the gingival margin in a coronal direction. In a gingival pocket, no destruction of the connective tissue fibers (gingival fibers) or alveolar bone occurs. This early

sign of disease in the mouth is completely reversible when the etiology of the edematous reaction is eliminated and frequently occurs without dental surgical therapy. However, in certain situations, a gingivectomy is necessary to reduce the gingival pocket depths to a healthy 1–3 mm.

As the original sulcular depth increases and the apical migration of the junctional epithelium has simultaneously occurred, the pocket is now lined by pocket epithelium (PE) instead of junctional epithelium (JE). [3] To have a true periodontal pocket, a probing measurement of 4 mm or more must be clinically evidenced. In this state, much of the gingival fibers that initially attached the gingival tissue to the tooth have been irreversibly destroyed. The depth of the periodontal pockets must be recorded in the patient record for proper monitoring of periodontal disease. Unlike in clinically healthy situations, parts of the sulcular epithelium can sometimes be seen in periodontally involved gingival tissue if air is blown into the periodontal pocket, exposing the newly denuded roots of the tooth. A periodontal pocket can become an infected space and may result in an abscess formation with a papule on the gingival surface. Incision and drainage of the abscess may be necessary, as well as systemic antibiotics; placement of local antimicrobial delivery systems within the periodontal pocket to reduce localized infections may also be considered. It is classified as supra bony and infra bony based on its depth in relation to alveolar bone. [4]

Leptin is a hormone predominantly made by adipose cells and enterocytes in the small intestine that helps to regulate energy balance by inhibiting hunger, which in turn diminishes fat storage in adipocytes. Leptin acts on cell receptors in the arcuate nucleus of the hypothalamus. Although regulation of fat stores is deemed to be the primary function of leptin, it also plays a role in other physiological processes, as evidenced by its many sites of synthesis other than fat cells, and the many cell types beyond hypothalamic cells that have leptin receptors. Many of these additional functions are yet to be defined. In obesity, a decreased sensitivity to leptin occurs (similar to insulin resistance in type 2 diabetes), resulting in an inability to detect satiety despite high energy stores and high levels of leptin. [5]

Factors that acutely affect leptin levels are also factors that influence other markers of inflammation, e.g., testosterone, sleep, emotional stress, caloric

restriction, and body fat levels. While it is well-established that leptin is involved in the regulation of the inflammatory response,[67][68][69] it has been further theorized that leptin's role as an inflammatory marker is to respond specifically to adipose-derived inflammatory cytokines.

In terms of both structure and function, leptin resembles IL-6 and is a member of the cytokine superfamily. Circulating leptin seems to affect the HPA axis, suggesting a role for leptin in stress response. Elevated leptin concentrations are associated with elevated white blood cell counts in both men and women. Similar to what is observed in chronic inflammation, chronically elevated leptin levels are associated with obesity, overeating, and inflammation-related diseases, including hypertension, metabolic syndrome, and cardiovascular disease. While leptin is associated with body fat mass, however, the size of individual fat cells, and the act of overeating, it is interesting that it is not affected by exercise (for comparison, IL-6 is released in response to muscular contractions). Thus, it is speculated that leptin responds specifically to adipose-derived inflammation. Leptin is a pro-angiogenic, pro-inflammatory and mitogenic factor, the actions of which are reinforced through crosstalk with IL-1 family cytokines in cancer. [6]

Taken as such, increases in leptin levels (in response to caloric intake) function as an acute pro-inflammatory response mechanism to prevent excessive cellular stress induced by overeating. When high caloric intake overtaxes the ability of fat cells to grow larger or increase in number in step with caloric intake, the ensuing stress response leads to inflammation at the cellular level and ectopic fat storage, i.e., the unhealthy storage of body fat within internal organs, arteries, and/or muscle. The insulin increase in response to the caloric load provokes a dose-dependent rise in leptin, an effect potentiated by high cortisol levels. (This insulin-leptin relationship is notably similar to insulin's effect on the increase of IL-6 gene expression and secretion from preadipocytes in a time- and dose-dependent manner.) Furthermore, plasma leptin concentrations have been observed to gradually increase when acipimox is administered to prevent lipolysis, concurrent hypocaloric dieting and weight loss notwithstanding. Such findings appear to demonstrate high caloric loads in excess of storage rate capacities of fat cells lead to stress responses that induce an increase in leptin, which then operates

as an adipose-derived inflammation stopgap signaling for the cessation of food intake so as to prevent adipose-derived inflammation from reaching elevated levels. This response may then protect against the harmful process of ectopic fat storage, which perhaps explains the connection between chronically elevated leptin levels and ectopic fat storage in obese individuals. [7]

Leptin increases the production of leukocytes via actions on the hematopoietic niche, a pathway that is more active in sedentary mice and humans when compared to individuals which are physically active. Orthodontics is a specialty of dentistry that deals with the diagnosis, prevention and correction of malpositioned teeth and jaws. It can also focus on modifying facial growth, known as dentofacial orthopedics. Abnormal alignment of the teeth and jaws is common, nearly 30% of the population has malocclusions severe enough to benefit from orthodontic treatment. Treatment can take several months to a few years, it involves the use of dental braces and other appliances to slowly move the teeth and jaws around. If the malocclusion is very severe, jaw surgery may be used. Treatment is usually started before a person reaches adulthood since bones can more easily be moved around in children.

A typical treatment for incorrectly positioned teeth (malocclusion) takes about 1 to 3 years to complete, with braces being altered slightly every 4 to 10 weeks by the specialists called orthodontists. Orthodontists are dental specialists who are University trained in the prevention, diagnosis and treatment of dental and facial irregularities. They provide a wide range of treatment options to straighten crooked teeth, fix bad bites and align the jaws correctly.[6] Multiple methods exist for adjusting malocclusion. In growing patients there are more options for treating skeletal discrepancies, either promoting or restricting growth using functional appliances, orthodontic headgear or a reverse pull facemask. Most orthodontic work is started during the early permanent dentition stage before skeletal growth is completed. If skeletal growth has completed, jaw surgery can be an option. Sometime teeth are extracted to aid the aid the orthodontic treatment (teeth are extracted in about half of all of cases, most commonly the premolars). [8]

Orthodontic therapy can include the used of fixed or removable appliances. The majority of orthodontic therapy is delivered using appliances that are fixed in

place, for example with braces that are bonded to the teeth with adhesives. Fixed appliances can have a greater mechanical control of the teeth and the treatment outcome is greater with the use of fixed appliances. Fixed appliances are for example used to rotate teeth that don't fit to the arch shape of the other teeth, to move multiple teeth to different places, to change the angle of teeth, or to change the position of the root of the tooth. It is not preferable if the patient has poor oral hygiene (as that can result in decalcification, tooth decay, and other problems), if the patient isn't motivated (as treatment lasts several months and commitment to oral hygiene is required), or if the malocclusions are mild. [9]

It is assumed that leptin has a role in protecting gingival tissues, leptin stimulates the immune system and enhances bone formation by acting directly on osteoblasts. As periodontal disease progresses, the protective role of leptin on the gingiva is lost owing to a decrease in the leptin level. During orthodontic tooth movement, the early response of periodontal tissues to mechanical stress is an acute inflammatory reaction. Study of leptin therefore is a useful guide to determine its relationship with tooth movement in both tension and pressure sites and the role of this cytokine in controlling the local inflammation around the tooth. Detection of the leptin level in GCF at sites under orthodontic movement had been tested and it was found that the concentration of leptin in GCF is decreased by orthodontic tooth movement.

Methodology:

The present study was planned in Department of Periodontology, Manipal College of Dental Sciences Manipal. Total 20 cases were evaluated in the present study. The 10 cases were enrolled in Group A as study cases and 10 cases were enrolled in control cases. The selected 10 patients were bonded with fixed appliance 0.022" PEA bracket slot, MBT prescription. The maxillary right canine (control tooth, CT) were not bonded with the bracket. The initial wire will be 0.016" NiTi wire. The distalisation force were applied on the left canine (test tooth, TT) using 0.010 SS lace backs.

Samples from 10 patients collected in the following manner:

On Test side: Samples collected at Initial and at 4, 8, 24 and 72 hours & 1 week after the orthodontic appliance on 10 maxillary left canine (TT).

On Control side: Samples collected at Initial and at 4, 8, 24 and 72 hours & 1 week after the orthodontic appliance on 10 maxillary right canine (CT).

GCF samples collected from the distal side of the upper right canines used as control tooth (CT) and left side canine as test tooth (TT) using a pipette without the contamination of blood and saliva. Isolate the tooth with cotton rolls, and supragingival plaque removed with a curette without touching the margin of gingiva. The sites will be gently dried with an air syringe and a saliva ejector put in place to avoid any salivary contamination. The GCF samples collected using microcapillary pipettes which are inserted into the crevices and left for 30secs. The pipettes with the collected GCF wrapped with thin aluminium foil and stored at -70 to -800 centigrade. Before the analysis, the samples centrifuged at 2,500g at 4o C. The results will be expressed in ng/mL. Reading performed at 450nm with a correction at 540nm to reduce optical imperfections on the reading plate.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study. Following was the inclusion and exclusion criteria for the present study.

Inclusion criteria:

1. Good general health status.
2. Cases requiring upper canine distalization with first premolar extraction.
3. Clinically and radiologically healthy periodontal tissues.
 - i. No gingival bleeding.
 - ii. Probing depth less than 3mm.
 - iii. No radiographic evidence of periodontal bone loss.
4. No antibiotic therapy in the past three months.
5. No use of anti-inflammatory drugs in the previous thirty days.
6. Good oral hygiene.
7. Non smoking.

Exclusion Criteria:

1. History of previous orthodontic treatment.
2. Subjects with known systemic disease or any other systemic disease which can alter the course of the periodontal disease.
3. Subjects having any autoimmune diseases.
4. Pregnant women.

Results & Discussion:

Gingival crevicular fluid (GCF) arises at the gingival margin and is otherwise termed transudate or exudate. The flow rate is related to the degree of gingival inflammation, and a rate of 0.05 to 0.20 μ L per minute was reported during minimal inflammation. Several studies have been performed on the composition of gingival crevicular fluid and the changes seen during orthodontic tooth movement. [10-11]

The mechanism of bone remodeling during orthodontic treatment is associated with the release of inflammatory mediators, such as prostaglandin-E2 and interleukin 1- β . [12] Neuropeptides such as substance P and Interleukin-1 β which are produced mainly by activated monocytes, initiate bone resorption [13] either by activating osteoclasts or by stimulating the synthesis of prostaglandin-E2. [14] Force applied to a tooth is known to cause the periodontal tissues to experience either tension or compression stress, depending on the tooth movement. [15] A variety of substances involved in the bone remodeling process are diffused into the gingival crevicular fluid. Therefore, gingival crevicular fluid sample analysis could help in understanding the ongoing biochemical processes associated with bone turnover during orthodontic tooth movement. [16]

The bone remodeling that occurs during orthodontic tooth movement is a biologic process involving an acute inflammatory response in the periodontal tissues. The sequence characterized by periods of activation, resorption, reversal, and formation has been recently described as occurring in both tension and compression tooth sites during orthodontic tooth movement. In Orthodontics, mechanical stress appears to evoke biochemical and structural responses in a variety of cell types in vivo as well as in vitro. The early phase of orthodontic tooth movement involves an acute inflammatory response, distinguished by periodontal vasodilation and leukocyte migration from the periodontal ligament capillaries. The mechanism of bone resorption could also be related to the release of inflammatory mediators found in the gingival crevicular fluid.

Leptin is a hormone predominantly made by adipose cells and enterocytes in the small intestine that helps to regulate energy balance by inhibiting hunger, which in turn diminishes fat storage in adipocytes. Leptin acts on cell receptors in the arcuate nucleus of the hypothalamus. Although regulation of fat stores

is deemed to be the primary function of leptin, it also plays a role in other physiological processes, as evidenced by its many sites of synthesis other than fat cells, and the many cell types beyond hypothalamic cells that have leptin receptors. Many of these additional functions are yet to be defined. In obesity, a decreased sensitivity to leptin occurs (similar to insulin resistance in type 2 diabetes), resulting in an inability to detect satiety despite high energy stores and high levels of leptin. [17]

Table 1: Levels of Gingival Crevicular Fluid Volume (mL)

	Cases Teeth	Control Teeth
Cases of	Orthodontic Treatment	Normal Teeth
Initial	0.54 – 1.18	0.55 – 1.28
At 4 hr	0.54 – 1.24	0.54 – 1.16
At 24 hrs	0.54 – 1.22	0.56 – 1.19
At 48 hrs	0.54 – 1.21	0.55 – 1.20
At 72 hrs	0.54 – 1.19	0.55 – 1.21
After 1 week	0.56 – 1.18	0.57 – 1.21p

Table 2: Levels of GCP Leptin (pg/mL)

	Control Teeth	Cases Teeth
Cases of	Normal Teeth	Orthodontic Treatment
Initial	1.78 – 2.91	1.27 – 3.31
At 4 hr	1.30 – 3.28	1.24 – 3.30
At 24 hrs	1.32 – 3.29	1.34 – 3.33
At 48 hrs	1.31 – 3.24	1.29 – 3.26
At 72 hrs	1.29 – 3.21	1.25 – 3.10
After 1 week	1.28 – 3.15	0.98 – 2.67

A study conducted to detect the presence of leptin in gingival crevicular fluid during orthodontic tooth movement and concluded that the concentration of leptin in GCF is decreased by orthodontic tooth movement; this study also suggests that leptin may have been one of the mediators responsible for orthodontic tooth movement. [18]

An in vitro study to evaluate the levels of pentraxin-3 in gingival crevicular fluid during orthodontic tooth movement suggested that there is Pentraxin-3 involvement in periodontal orthodontic remodeling of young and adult patient. [19]

An in vitro study to compare and evaluate the levels of pentraxin-3 in gingival crevicular fluid and plasma in periodontal health and disease, found out a positive correlation between the both. Based on the present study, GCF PTX-3 values were considered as a marker of inflammatory activity in periodontal disease.3= [20]

A study was conducted to evaluate the total concentration of GCF with variation in sampling timing and gingival inflammation and concluded that there is an increase in the proportion of serum derived molecule in the more proteinaceous GCF sample. This suggested the extreme sensitivity of the gingival vasculature to the GCF sampling and consequently the need for accurate standardization of GCF collection protocol. [21]

A study was conducted to investigate whether the GCF is an inflammatory exudate and concluded that some irritation whether chemical or mechanical was necessary to induce the production of GCF and therefore, it should be considered as a pathological phenomenon. Thus, the flushing effect of GCF has concluded that GCF is an important component of the protective mechanism of the crevicular region. [22]

A study conducted on the elevated C-reactive protein (CRP) in the Gingival Crevicular Fluid (GCF) concluded that C- reactive protein in GCF appears to be of systemic origin and therefore maybe indicative of systemic inflammation from either a periodontal infection or inflammatory disease elsewhere. [23]

Dilsiz et al., who evaluated leptin concentration in GCF before and after force application up to one week, reported a gradual decrease in GCF leptin concentration which is contradictory to our results. [24] The results in this study was also different from previous studies that measured other biomarkers during orthodontic tooth movement, where an exponential increase in concentrations of the mediators was observed after force application.

In this study, there were changes in GCF leptin concentration during orthodontic force application, and a positive correlation between rate of tooth movement and leptin concentration. Future long-term evaluation of leptin concentration during orthodontic tooth movement can reveal additional information. Also, relating leptin concentration to levels of other important cytokines during orthodontic tooth movement would help us find the specific role of leptin in orthodontic tooth movement.

Conclusion:

Significantly decreased levels of leptin concentration might result from the presence of inflammation adjacent to the teeth undergoing movement. It has been shown previously that orthodontic tooth movement may therefore show local traits of a damage/ repair process with inflammation-like

reactions: high vascular activity, many leukocytes and macrophages, and involvement of the immune system.

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